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# Inducible Interleukin-1 Gene Expression in Human Vascular Smooth Muscle Cells

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## Abstract

Interleukin-1 (IL-1) mediates many components of generalized host response to injury and may also contribute to local vascular pathology during immune or inflammatory responses. Because altered function of smooth muscle cells (SMC) accompanies certain vascular diseases, we tested whether SMC themselves might produce this hormone. Unstimulated SMC contain little or no IL-1 mRNA. However, exposure to bacterial endotoxin caused accumulation of IL-1 mRNA in SMC cultured from human vessels. Endotoxin maximally increased IL-1 $\beta$  mRNA in SMC after 4–6 h. The lowest effective concentration of endotoxin was 10 pg/ml. 10 ng/ml produced maximal increases in IL-1 $\beta$  mRNA. Interleukin-1 $\alpha$  mRNA was detected when SMC were incubated with endotoxin under “superinduction” conditions with cycloheximide. Endotoxin-stimulated SMC also released biologically functional IL-1, measured as thymocyte costimulation activity inhibitable by anti-IL-1 antibody. Thus, human SMC can express IL-1 $\beta$  and IL-1 $\alpha$  genes, or very similar ones, and secrete biologically active product in response to a pathological stimulus. Endogenous local production of this inflammatory mediator by the blood vessel wall’s major cell type could play an important early role in the pathogenesis of vasculitis and arteriosclerosis.

## Introduction

It is increasingly apparent that the blood vessel is not a passive conduit, but a complex organ composed of highly differentiated cells with specialized functions that play important roles in health and disease. The endothelial cell that lines the luminal surface of the vessel wall has recently received much attention in this regard. Blood-borne signals can modulate several important vascular endothelial cell functions related to thrombosis, interaction with leukocytes, and immune competence (1–7). Endothelial cells can also secrete substances that influence the contractile or growth properties of smooth muscle cells (SMC)<sup>1</sup> that

comprise the tunica media of the vessel (8–10). Although the vascular SMC is by far the most numerous cell type in the vessel wall, its repertoire of inducible responses has received very little attention. The SMC is generally regarded as a target for secretory products of endothelial cells, platelets, and leukocytes, rather than as a source of signals that control the function of other cell types (10).

There is some evidence that SMCs themselves may elaborate mediators involved in the pathogenesis of vascular diseases. Inbred mice of the MRL/lpr strain develop a spontaneous autoimmune vasculitis. In contrast to vascular SMCs from normal mice, those from arteries of MRL/lpr mice express I-region-associated (Ia) antigens and release an interleukin 1–like thymocyte activating activity (11). These altered immune functions of vascular SMCs probably contribute to the localized pathology in these mice with genetically determined vasculitis.

Interleukin 1 (IL-1), formerly thought to be produced primarily by cells of the monocyte/macrophage lineage, mediates several key components of systemic acute phase response (12–14). Certain biological properties of IL-1, such as its abilities to act as a chemoattractant and degranulating agent and to induce synthesis of several proteins, including collagen and proteinases, support the concept that IL-1 is also involved in the development of local lesions associated with tissue injury (e.g., synovitis, hepatic or pulmonary fibrosis). In the context of the blood vessel, IL-1 can induce several pathogenic functions of vascular endothelial cells, such as synthesis of a tissue-factor–like procoagulant activity and increased leukocyte adhesion (2–4).

Secretion of IL-1 by SMCs themselves might be very important not only in the vasculitides, but in the early stages of atherogenesis as well. The capacity of human vascular SMCs to produce IL-1 activity has not yet been investigated. We report here studies of the regulation of IL-1 gene expression in passaged primary cultures of vascular SMCs obtained from human adults. These experiments identify this cell type as an unexpected source of this important mediator that may play a significant role in vascular pathology.

## Methods

**Cell preparations and culture.** Human SMCs were grown from explants or enzymatic digests of surgical specimens of aorta or from trimmed portions of the tunica media of saphenous veins after enzymatic removal of endothelial cells (15, 16). The use of excess portions of human vascular tissue obtained at surgery was approved by the Human Investigation Review Committee of the New England Medical Center. Cell cultures prepared in this manner exhibit typical morphology and “hill and valley” growth pattern of cultured vascular SMCs (17). These cells were maintained in Dulbecco’s modified Eagle’s medium (Irvine Scientific, Santa Ana, CA) buffered with Hepes (25 mM), and supplemented with fetal bovine serum (10%; HyClone Laboratories, Logan, UT). These cells were subcultured and harvested as previously described (15, 16). An isolate of cell line U-937 derived from human histiocytic lymphoma was pur-

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1. **Abbreviations used in this paper:** Ia, I-region-associated; IL-1, interleukin 1; kb, kilobase; PHA, phytohemagglutinin; pI, isoelectric points; SMC, smooth muscle cell.

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chased from the American Type Culture Collection, Rockville, MD, and was maintained and passed as recommended.

**Sources, preparation, and labeling of DNA probes.** There are two known human genes that encode molecules with IL-1 activity. The products of these genes share only 26% homology of amino acid sequence and have distinct isoelectric points (pI; 18, 19). IL-1 $\beta$  has a pI of 7 and is the major form expressed by human monocytes (12, 18). IL-1 $\alpha$  has a pI of 5 and is the less abundant species of IL-1 mRNA in activated human mononuclear phagocytes (19). The IL-1 $\beta$  probe used in these studies was an Aha III-to-FnuD II fragment (1.1 kilobases [kb]) of a full-length cDNA clone (pCD-418) that encodes the precursor for this form (18). For the alpha species of human IL-1 (pI, 5) we used a synthetic oligonucleotide probe (positions 298–339) that was end-labeled with gamma [ $^{32}$ P]dATP to specific activities  $>10^9$  dpm/ $\mu$ g DNA (19, 20).

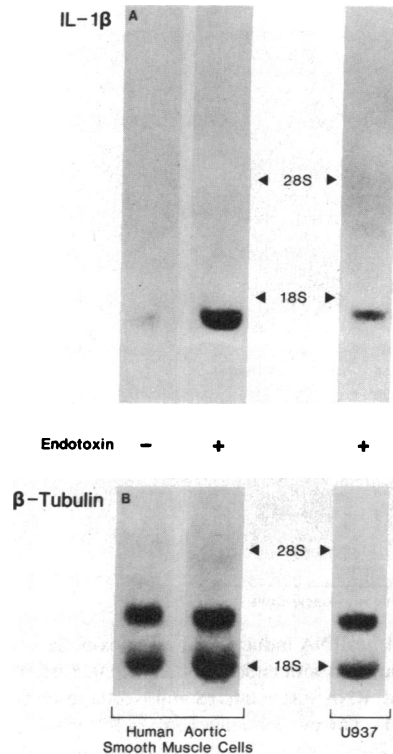
The  $\beta$ -tubulin probe used was a Pst I-to-Bam HI fragment (1.3 kb) of a rat  $\beta$ -tubulin cDNA clone (RBT.3) that is expressed constitutively in many tissues (21). This fragment contains most of the translated region of this clone and is highly conserved between species. Plasmids were isolated chromatographically (22). The double-stranded probes were labeled with [ $^{32}$ P]dCTP and [ $^{32}$ P]dGTP by nick translation to specific activities  $>10^8$  dpm/ $\mu$ g DNA (20).

**Extraction of RNA and Northern analysis.** Total cellular RNA was isolated by the guanidinium isothiocyanate/cesium chloride method (20). The concentration of RNA was determined by absorbance at 260 nm. The ratios of 28 S:18 S RNA and of A 260/A 280 were greater than 2:1. For Northern hybridization analysis, RNA was electrophoresed into agarose gels (1.2%) that contained formaldehyde (2.2 M). After capillary transfer to nylon membranes (Hybond-N, Amersham Corp., Arlington Heights, IL), the nucleic acids were immobilized by short-wave UV irradiation. We used standard techniques for prehybridization, hybridization, and autoradiography (20). Hind III fragments of phage lambda DNA were used as size markers. Quantitative analysis of autoradiograms from Northern blots required use of a soft laser densitometer (Ultrosan 2202; LKB Instruments, Gaithersburg, MD) equipped with a recording integrator (HP 3390A). The results presented represent arbitrary units that correspond to the relative areas under the densitometric peaks.

**Biological determination of interleukin-1 activity.** Biologically functional IL-1 was measured as the ability to increase the proliferation of thymocytes incubated with a suboptimal concentration of the mitogenic lectin phytohemagglutinin (PHA). The ability to complement PHA-induced mitogenesis is known as thymocyte costimulation, and is characteristic of IL-1 (23, 24). Thymocytes from C3H/HeJ mice (Jackson Laboratory, Bar Harbor, ME) were incubated for 72 h in RPMI 1640 medium supplemented with fetal calf serum (10%), PHA (1  $\mu$ g/ml), with or without test specimens. Tritiated thymidine (6–7 Ci/mM, 10  $\mu$ Ci/ml) was added during the last 18 h of this incubation, and the radioactivity incorporated was determined by liquid scintillation spectroscopy. Interleukin-1 $\beta$  produced by recombinant DNA techniques or purified from human blood monocytes was used as a positive control. Triplicate determinations were performed on each sample, and results are presented as mean  $\pm$  standard deviation.

## Results

**Human vascular SMCs express the IL-1 $\beta$  gene in response to bacterial endotoxin.** Human vascular SMCs cultured under the usual conditions contained little or no messenger RNA (mRNA) that hybridized with the IL-1 $\beta$  probe (Fig. 1). Endotoxins obtained from Gram-negative bacteria are potent inducers of IL-1 production by mononuclear phagocytes (12–14). Accordingly, we tested whether this pathologic stimulus would induce expression of the IL-1 $\beta$  gene in adult human aortic SMCs. Bacterial endotoxin increased the proportion of IL-1 $\beta$  message in RNA extracted from these cells in two independent experiments (Fig. 1 and data not shown). This phenomenon was also observed in



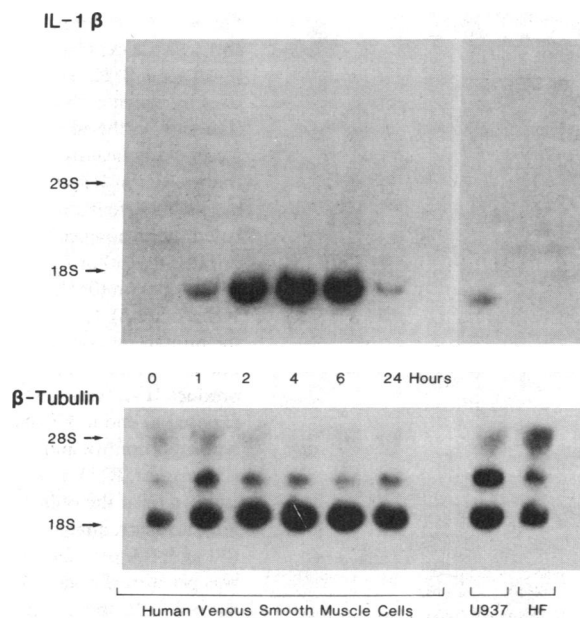
**Figure 1.** Adult human aortic SMCs express the IL-1 $\beta$  gene. SMCs isolated by enzyme dissociation from the aorta of a previously healthy trauma victim (14-yr-old male) were incubated in the absence (–) or presence (+) of *E. coli* endotoxin (055:B5; 10  $\mu$ g/ml; 6 h). Cells of the human monocytoid line U-937, known to produce IL-1, were also exposed to endotoxin as a positive control and size marker. RNA was isolated from the cells, and Northern analysis (20  $\mu$ g RNA per lane) was performed as described in Methods. The positions of the 28 S and 18 S ribosomal subunits visualized by ultraviolet light are indicated in this and subsequent northern blots. A shows

a hybridization of this blot with a nick-translated IL-1 $\beta$  probe. The IL-1 $\beta$  mRNA from the endotoxin-stimulated smooth muscle and U-937 cells comigrate. Analysis of this and other Northern blots calibrated with DNA standards indicated that the size of this mRNA was  $\sim$ 1.6 kb. B shows a rehybridization of the same Northern blot with the  $\beta$ -tubulin probe after removal of the IL-1 probe. The two transcript sizes found in these cells are similar to those constitutively expressed in many other tissues.

cells derived from medial explants of human saphenous vein (Fig. 2). The size of the transcript was  $\sim$ 1.6 kb, and this mRNA species migrated closely with IL-1 transcripts extracted from endotoxin-stimulated human monocytes or from U-937 cells (Figs. 1 and 2, and data not shown).

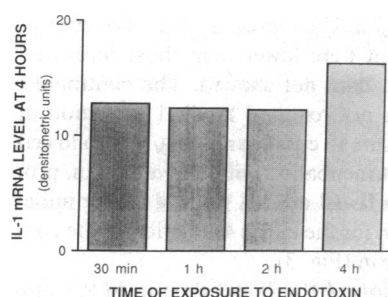
The accumulation of IL-1 $\beta$  mRNA in human SMCs depended on the length of incubation with endotoxin. Exposure of human saphenous vein SMCs to endotoxin increased IL-1 $\beta$  message levels substantially after just 1 h (Fig. 2). In two independent experiments, IL-1 $\beta$  mRNA levels after 24 h of continuous exposure to endotoxin were lower than those measured after 4 or 6 h (Fig. 2 and data not shown). The continuous presence of endotoxin was not required for IL-1 induction in these cells. 30 min of exposure to endotoxin (1  $\mu$ g/ml), followed by washing and subsequent incubation without endotoxin, produced a similar increase in IL-1 $\beta$  mRNA levels 4 h after initial exposure, as did incubation for the entire 4-h period in the continued presence of endotoxin (Fig. 3).

The increased expression of the IL-1 $\beta$  gene in SMCs also depended on the concentration of endotoxin. Concentrations as low as 10 pg/ml increased IL-1 $\beta$  mRNA levels severalfold (Fig. 4). Polymyxin B, an antagonist of the actions of endotoxin mediated by the lipid A moiety, inhibited this response (Fig. 4). This result confirmed that the ability of endotoxin preparations



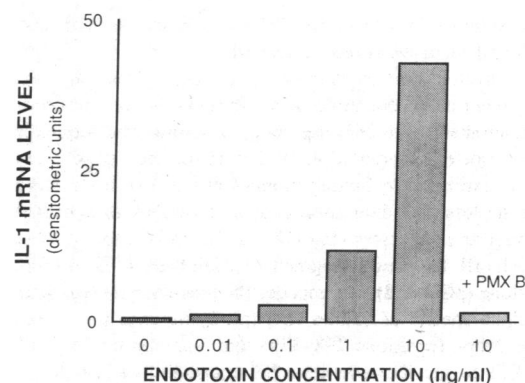
**Figure 2.** Time course of IL-1 $\beta$  mRNA induction by endotoxin. Saphenous vein SMCs were incubated with endotoxin (*E. coli*; 055:B5, 1  $\mu$ g/ml) for the times indicated. RNA was extracted and Northern blotting was performed (see Fig. 1). The two left lanes contain RNA extracted from U-937 cells (see Fig. 1) or human dermal fibroblasts. The upper panel shows the results of a hybridization with the IL-1 $\beta$  probe, the bottom panel shows a rehybridization with the  $\beta$ -tubulin probe (see Fig. 1).

to augment levels of IL-1 mRNA was due to the lipopolysaccharide and not contaminating material. The ability of bacterial lipopolysaccharides to augment IL-1 $\beta$  gene expression was not limited to products of one species, serotype, or Gram-negative organism. Endotoxin from *E. coli* 0111:B4 (L-2630; Sigma Chemical Co., St. Louis, MO), *Salmonella typhosa* (L-6386; Sigma Chemical Co.) or *Salmonella minnesota* (L-6261; Sigma Chemical Co.) all increased IL-1 $\beta$  mRNA content in SMCs (Fig. 5). The marked increase in IL-1 $\beta$  gene expression in endotoxin-stimulated human SMCs was not due to a non-specific effect on mRNA levels, since rehybridization of a number of Northern blots with  $\beta$ -tubulin or  $\alpha$ -actin probes showed no consistent change in the level of these mRNAs under these conditions (Figs. 1, 2, 5 and 6; bottom panels, and data not shown).



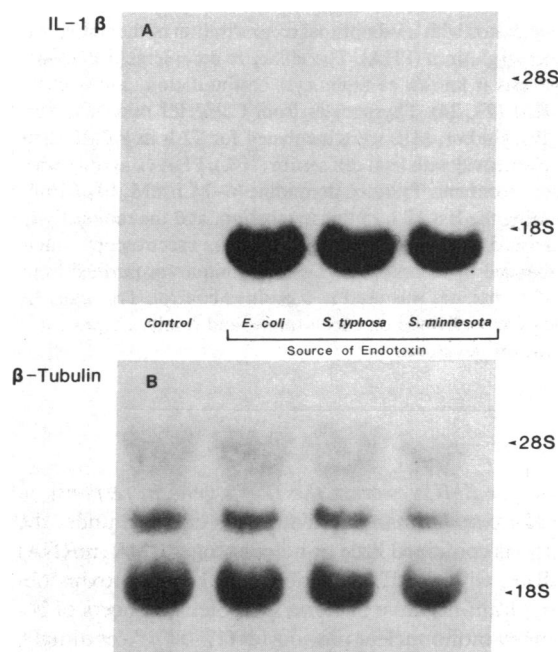
**Figure 3.** Maximal induction of IL-1 $\beta$  mRNA in SMCs does not require continued presence of endotoxin. Human saphenous vein SMCs were incubated with or without endotoxin (*E. coli*; 055:B5, 1  $\mu$ g/ml). After exposure to endotoxin for the times indicated, the cells

were washed in medium containing the endotoxin antagonist polymyxin B (10  $\mu$ g/ml), and subsequently incubated in medium lacking endotoxin. After incubation for a total of 4 h, RNA was isolated from each culture, and the amount of IL-1 $\beta$  mRNA was determined by Northern blotting.

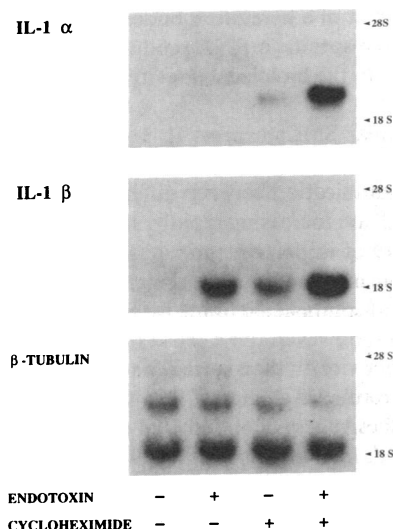


**Figure 4.** Concentration dependence of endotoxin induced IL-1 $\beta$  expression in human saphenous vein SMCs. Cultures of human saphenous vein SMCs were exposed to the indicated concentrations of endotoxin (*E. coli*; 055:B5) for 4 h. A duplicate culture incubated with the highest concentration tested also contained the endotoxin antagonist polymyxin B (PMX B, 1  $\mu$ g/ml). The results are presented in arbitrary units derived by quantitative densitometry of the autoradiogram of a Northern blot hybridized with the IL-1 $\beta$  probe.

**Detection of IL-1 $\alpha$  mRNA in SMCs "superinduced" in the presence of cycloheximide.** Under usual conditions, no IL-1 $\alpha$  mRNA was detected in SMCs even after incubation with endotoxin under conditions that readily induced IL-1 $\beta$  mRNA (Fig. 6 and data not shown). In SMCs incubated with cycloheximide (10  $\mu$ g/ml), IL-1 $\beta$  mRNA increased and IL-1 $\alpha$  mRNA was detected (Fig. 6). Simultaneous incubation with cycloheximide and endotoxin increased mRNA for both of these human IL-1 genes substantially (Fig. 6). This brief incubation with cy-



**Figure 5.** Various bacterial endotoxins induce IL-1 $\beta$  mRNA in human SMCs. Human saphenous vein SMCs were incubated without (Control), or with endotoxins (1  $\mu$ g/ml) from the three bacterial species indicated for 6 h. Northern analysis and presentation of the data are as in Fig. 1.



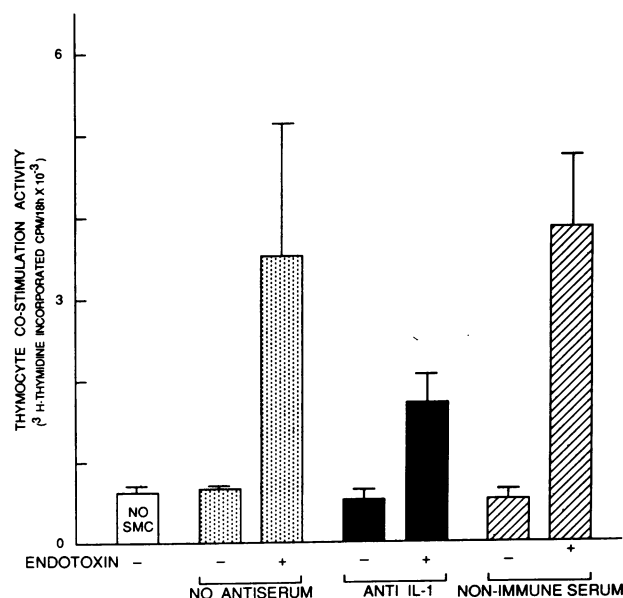
**Figure 6.** "Superinduction" of IL-1 $\alpha$  and IL-1 $\beta$  mRNA in human vascular SMCs. Human saphenous vein SMC cultures were incubated without (–), or with (+) cycloheximide (10  $\mu$ g/ml) for 2 h. After this 2-h preincubation, the cultures were incubated without (–), or with (+) endotoxin (*E. coli*; 055:B5, 1  $\mu$ g/ml) for another 2 h. In the cycloheximide (+) conditions, the cycloheximide was also included in the test incubation with or without endotoxin. RNA was extracted from all cultures after incubation for a total duration of 4 h (including the 2-h preincubation with or without cycloheximide). Northern analysis was performed as in Fig. 1. The top panel shows the result of a hybridization with the IL-1 $\alpha$  probe. The middle panel shows a hybridization of the same Northern blot with the IL-1 $\beta$  probe. The bottom panel shows a third hybridization of the same blot with the  $\beta$ -tubulin probe.

cycloheximide did not alter the viability of the cells as determined by phase microscopic observation or cause a decline in the number of cells harvested for RNA extraction. The total RNA content per cell was likewise unchanged by the 4-h incubation with cycloheximide (data not shown).

**Biologically active IL-1 is secreted by vascular SMCs exposed to endotoxin.** To determine if SMCs stimulated by endotoxin also produce and secrete functional IL-1, we examined whether material elaborated by these cells exhibited thymocyte costimulation, a characteristic biological activity of IL-1. In the absence of endotoxin, human saphenous vein or aortic SMCs secrete little or no IL-1 detected by this assay (Tables I and II, Fig. 7). Medium collected from endotoxin-stimulated SMCs secreted biological activity that was detected over a range of concentrations tested (Table I). Thymocyte costimulation activity released by stimulated saphenous vein SMCs was partially neutralized by rabbit antihuman monocyte IL-1 antibody diluted 1:100 (Fig. 7, *solid bars*). The same dilution of nonimmune serum did not reduce this activity (Fig. 7, *crosshatched bars*). In an independent experiment, human aortic SMCs treated with endotoxin released thymocyte costimulation activity that was completely neutralized by the anti-IL-1 antibody (Table II).

## Discussion

The vascular SMC is an unexpected and novel source of IL-1, an important inflammatory mediator previously thought to be produced primarily by mononuclear phagocytes. Recently, pro-



**Figure 7.** Human venous SMCs can secrete biologically active IL-1. Human saphenous vein SMCs were incubated without (–), or with endotoxin (*E. coli*; 055:B5; 1  $\mu$ g/ml) for 24 h. The supernatant media were assayed for thymocyte costimulation activity (see Methods). Data shown are mean $\pm$ SD of three to six replicate determinations on media from one culture. The value measured using medium that had not been exposed to SMCs is shown by the open bar. The stippled bars show activity measured in the absence of antibody. The solid bars show data measured when rabbit antihuman blood monocyte IL-1 (1:100) was included during the thymocyte assay. The cross-hatched bars show data obtained when this assay was performed in the presence of nonimmune rabbit serum (1:100).

duction of IL-1-like activity has been attributed to other cells that are not derived from bone marrow (3, 25–30). For example, human keratinocytes can produce a thymocyte-activating factor that resembles IL-1 (25). Astrocytes and oligodendroglia are another potential source of such activity (26). In addition, several laboratories have observed IL-1-like activity secreted by B lymphocytes, epithelial and mesangial cells, and vascular endothelial cells (3, 7, 27–30). We are unaware of previous descriptions of such activity from human vascular SMCs.

**Table I.** Endotoxin-stimulated Human Aortic SMCs Secrete Biologically Active IL-1

		Thymocyte costimulation activity	
	Concentration	–Endotoxin	+Endotoxin*
Medium from human aortic SMCs	1:2	11,348 $\pm$ 2,001	26,761 $\pm$ 6,052
	1:4	9,227 $\pm$ 521	20,374 $\pm$ 1,823
	1:8	7,893 $\pm$ 1,479	21,672 $\pm$ 2,975
	1:16	4,078 $\pm$ 3,393	18,445 $\pm$ 3,806
Monocyte IL-1‡	1 U/ml	28,458 $\pm$ 4,295	

Data are thymidine incorporation in cpm (mean $\pm$ SD,  $n$  = 3). The background incorporation induced by PHA alone in this experiment was 7,809 $\pm$ 2,384.

\* Medium was conditioned by the SMCs in the presence of endotoxin (*E. coli*, 055:B5, 10  $\mu$ g/ml) for 24 h.

‡ IL-1 purified as previously described (24).

Table II. Anti-IL-1 Antibody Neutralizes IL-1 Activity Released by Endotoxin-stimulated Human Aortic Smooth Muscle Cells (HASMC)

		Thymocyte costimulation activity	
		Concentration	
		-Anti-IL-1	+Anti-IL-1*
Medium from HASMC	1:10	5,697±1,600	3,585±361
Medium from endotoxin-stimulated HASMC†	1:10	8,079±840	3,214±336
Recombinant IL-1β	200 ng/ml	24,688±5,863	3,717±128

Data are thymidine incorporation in cpm (mean±SD, *n* = 3). The background incorporation induced by PHA alone in this experiment was 4,021±677.

\* Rabbit anti-monocyte-derived IL-1 antibody was added at 1:100 dilution.

† Endotoxin exposure was to *E. coli* 055:B5 (10 µg/ml) for 24 h.

Incubation of SMCs with cycloheximide increased the levels of IL-1α and IL-1β mRNA, and accentuated the endotoxin-induced increases in these transcripts (Fig. 6). This phenomenon, known as "superinduction," may be due to increased transcription and/or reduced rate of mRNA catabolism (31, 32). Increased steady-state IL-1 mRNA levels under conditions that inhibit protein synthesis is consistent with modulation of IL-1 gene expression by a labile intracellular protein. The failure to detect IL-1α sequences in SMC RNA in the absence of cycloheximide may indicate that, as in the human monocyte, this species of IL-1 is a less abundant form. Dayer et al. recently reported that activated human blood monocytes contain at least 50-fold more mRNA for IL-1β than for IL-1α (33). The lower steady-state levels of IL-1α relative to IL-1β mRNA detected in the SMCs might also be due to differences in the stability of the specific messages, the efficiency of detection with probes, or hybridization conditions employed. However, the specific activity of the end-labeled IL-1α probe was actually substantially higher than that of the nick-translated IL-1β cDNA probe (see Methods), and the end-labeled probe readily detected IL-1α sequences in RNA isolated from human mononuclear cells exposed to endotoxin without cycloheximide (data not shown).

The RNA species isolated from SMCs that hybridized with the IL-1 DNA probes used in this study had similar electrophoretic mobility to those found in human blood monocytes and U-937 cells (18, 19, and Figs. 1 and 2). Furthermore, results of preliminary Southern analysis of restriction digests of human genomic DNA show no evidence for multiple IL-1β genes (Ordovas, J. M., and P. Libby, unpublished observations). These observations suggest that IL-1 secreted by endotoxin-stimulated SMCs is produced by the same IL-1 genes previously cloned from human monocytes. The results presented here did not explore the biochemical nature of IL-1 activity secreted by SMCs. Preliminary studies indicate that SMC IL-1 may differ in molecular weight and immunoreactivity from fully processed IL-1 secreted by human blood monocytes (Warner, S. J. C., and P. Libby, unpublished observations). Thus, the posttranslational modification of SMC-derived IL-1 may be distinct. This might explain why the concentration of anti-IL-1 antibody tested did not completely neutralize thymocyte costimulation activity secreted by SMCs in all experiments (Fig. 7). However, our data

do not exclude that a product of a heretofore undescribed IL-1 gene or genes, or that a tissue-specific mRNA produced by exon shuffling, could contribute to the biological activity released by the SMCs.

Our finding that vascular SMCs express IL-1 genes in response to a pathological stimulus has several important implications. Examples of communication between different types of cell in the blood vessel wall are increasing rapidly. IL-1 has profound effects on a number of important functions of vascular endothelial cells. Under normal conditions vascular endothelial cells provide the ideal nonthrombogenic lining for blood vessels (34). When exposed to IL-1, however, these cells express a tissue factor-like procoagulant activity on their surface and could thus actually promote local thrombosis at sites of IL-1 release (2, 3, 34). IL-1 increases the adhesiveness of endothelial cells for leukocytes of all classes tested, including granulocytes and monocytes (4). Exposure to this cytokine also causes a striking shape change of human endothelial cells in culture, probably due to reorganization of the cytoskeleton (35). IL-1 alters the pattern of prostanoïd synthesis by endothelial cells (36, 37). These and other activities of IL-1 on endothelial cells have already been confirmed using recombinant IL-1α and IL-1β (38, 39).

Because of these manifold effects of IL-1 on endothelial function, the production and secretion of this mediator by the underlying SMCs might provide a novel example of interaction between neighboring cell types in the vascular wall. Information transfer between adjacent cells of different types has been termed "paracrine" control (40). There are already a number of examples of potential paracrine regulation of SMCs by products of endothelial cells (8–10). The present data suggest that information may also flow in the opposite direction, from SMCs to the endothelium.

Studies on the MRL/lpr mouse provide an example of how IL-1 production by SMCs might be involved in vascular disease. These mice develop a spontaneous autoimmune arteritis, characterized by infiltration of mononuclear cells, followed by cytolytic injury to arterial SMCs. Moyer and Reinisch found that SMCs isolated from the media of these mice expressed Class II major histocompatibility complex determinants (Ia molecules), and thus might function as antigen-presenting cells. In addition, they found that SMCs cultured from arteries of MRL/lpr mice, but not from congenic C3H mice, secreted IL-1-like biological activity (11). These studies on vasculitic mice support the potential in vivo relevance of the present observations on cultured human vascular SMCs (11).

Since the characteristic biological activities of IL-1 include chemotaxis and activation of granulocytes and T-cells, local production of IL-1 is likely involved in the pathogenesis of vascular inflammation in other situations. By increasing the expression of leukocyte adherence molecules on the surface of neighboring endothelial cells, IL-1 secreted by SMC could recruit leukocytes to sites of vascular injury or inflammation (4, 5). Further secretion of IL-1 and other mediators by these infiltrating leukocytes could amplify the original local signal furnished by IL-1 from vascular wall cells themselves, and perpetuate the inflammatory reaction.

The present study focused on bacterial endotoxins as stimuli that induce IL-1 production by SMCs. Other factors may also signal the production of this mediator by these cells. Such stimuli might include antigen-antibody complexes, mitogens such as platelet-derived growth factor, oxidized lipoproteins that are known to injure SMCs, or even mechanical factors such as altered

stress in the blood vessel wall due to local hemodynamic abnormalities. Although endotoxins cause lytic injury to some cultured cells, phase-contrast microscopic examination disclosed no morphologic sign of cytotoxicity due to these substances in the current studies. In addition, levels of  $\beta$ -tubulin and  $\alpha$ -actin mRNAs did not change substantially in endotoxin-treated SMCs. Thus, the effect of endotoxin on IL-1 gene expression in human SMCs did not appear to result from a nonspecific toxic effect.

The results presented here are consistent with the current concept that cells of the blood vessel wall are active participants in the maintenance of normal function and in the pathogenesis of vascular diseases. The finding that perturbed vascular SMCs can produce IL-1 demonstrates that this cell type can provide a primary stimulus for local inflammatory or immune response in the blood vessel wall. Although the present data establish that human SMCs can produce IL-1 in vitro, further study will be required to test whether these cells express this gene in vivo under pathologic conditions, and to determine the range of biologically relevant inducers of this phenomenon.

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